King County Marine Phytoplankton Monitoring Program

Sampling and Analysis Plan

August 2008

King County

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NAME OF PROJECT: Marine Phytoplankton Monitoring Program
PROJECT NUMBER: **421250**

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1.0 INTRODUCTION

Phytoplankton (microscopic plants) are the foundation of the food web in marine ecosystems. They are grouped into several broad categories, with diatoms, dinoflagellates, and coccolithophorids being the most common large phytoplankton. Their productivity and community structure impact all other organisms directly or indirectly as they provide food for zooplankton and fish, absorb and scatter light, and release dissolved organic matter. Little is known regarding Puget Sound phytoplankton community structure, particularly with regard to inter-annual variability and in response to water quality and climate changes (both short and long term). The resources currently designated for phytoplankton research in Puget Sound are almost entirely focused on issues surrounding toxic algae. Without a knowledge base of Puget Sound phytoplankton community structure and abundance, it will be difficult to predict how changes in climate and other regional stressors will impact the food web. The Puget Sound Partnership was formed in 2007 in order to achieve a healthy Puget Sound by 2020 and King County developed a Climate Plan in order to address and reduce impacts resulting from climate change. Phytoplankton are an integral part of a healthy Puget Sound.

Phytoplankton are highly sensitive to changes in nutrient concentrations but are also affected by hydrologic and climate changes. The primary effect of nutrient enrichment is an increase in primary production. Primary production is difficult to measure as it requires specialized equipment, including the use of a radioactive tracer. The quantitative result of primary production is phytoplankton biomass, which is a measure of the amount of chlorophyll-*a*. Although chlorophyll-*a* is not an exact measure of biomass, it is widely used as a gauge for biomass and it is relatively easy to measure. The assessment of chlorophyll-*a* together with phytoplankton community structure provides information that can be used to address the goals and objectives listed below.

1.1 MONITORING PROGRAM GOALS AND OBJECTIVES

King County's ongoing marine monitoring program includes analysis of physical, chemical, and biological (chlorophyll-*a*) water quality parameters at various locations throughout the Puget Sound Central Basin. However, the monitoring program does not include phytoplankton community structure or abundance.

Obtaining a long-term phytoplankton community dataset is critical to providing the ability to explain biological response with regard to environmental change. Given the limited temporal and spatial sampling resolution allowed by available resources, capturing phytoplankton response to nutrients and other factors will be difficult as phytoplankton response can occur on the scale of a day. However, the proposed monitoring program described in the following sections will provide information directly relevant to phytoplankton structure and nutrient changes over time. The proposed phytoplankton monitoring will achieve the following objectives:

- assess relative abundance and community composition of the major phytoplankton taxonomic groups at three locations within the Central Basin during the bloom season,
- document timing of seasonal shifts in major taxonomic groups (e.g., shifts from diatoms to dinoflagellates),

- detect long-term changes in taxonomic composition at the family level

Shifts in phytoplankton community composition can be indicative of anthropogenic stress locally and globally (e.g. climate change). Initially, this phytoplankton monitoring program will be limited in size and scope due to available resources and the need to establish standard protocols. However, in the long-term, King County's goal is to expand the phytoplankton monitoring program and build a more extensive database that could be used to answer ecosystem level questions such as, "Are environmental or anthropogenic changes negatively impacting the lower trophic levels in the Puget Sound Central Basin food web?".

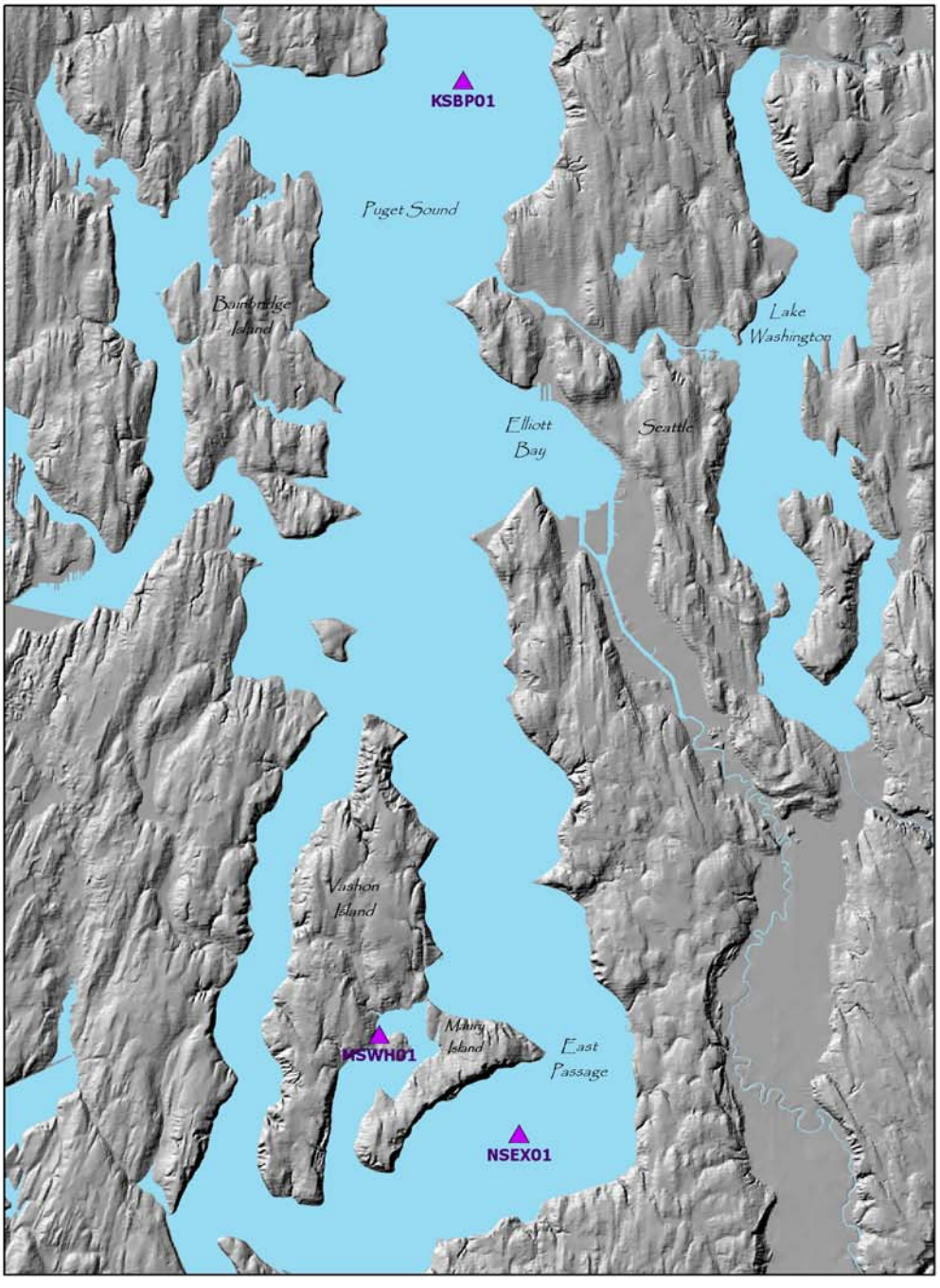
2.0 SURVEY DESIGN

Marine phytoplankton community structure and associated physical and chemical water quality parameters, including chlorophyll-*a*, will be collected synoptically from select stations within Puget Sound. Both physical and chemical factors can greatly influence phytoplankton abundance and distribution. All unique taxa will be identified and the qualitative abundance of each will be estimated from a sample collected at maximum chlorophyll-*a* depth. The details of the study design are described in this section.

2.1 STATION LOCATIONS

Three stations were identified for the initial phase of marine phytoplankton monitoring (Figure 1). Two stations are long-term marine program ambient monitoring stations, East Passage (NSEX01) and Pt. Jefferson (KSBP01), and were selected to provide north and south spatial coverage within King County waters in the Puget Sound Central Basin. The third station, located within the inner harbor of Quartermaster Harbor (MSWH01), has been part of the marine ambient monitoring program since 2006 and a telemetered mooring that provides high-frequency water quality data was installed in late 2007. This site was selected due to unique physical and chemical characteristics. Quartermaster Harbor is a shallow, protected embayment comprised of an inner and outer harbor that has poor tidal flushing. As a result, water quality, particularly dissolved oxygen and nitrogen compounds, is a concern.

Figure 1. Station Map



2.2 SAMPLING FREQUENCY

The marine phytoplankton monitoring program will begin in April 2008 with sampling events at all three stations twice monthly. Sampling will occur only during the spring and summer bloom season April through September each year. The first sampling event will be conducted during the first 5-day work week of each month and the second sampling event will co-occur during the regular ambient offshore monthly sampling in the third week of each month. Because the Quartermaster Harbor station is not sampled by boat as are the other stations, sampling at this station will be conducted from land and separately from the monthly ambient sampling. Sample collection at all stations will be completed within the hours of 8 am and 4 pm to capture diurnal migrants in the photic zone.

2.3 DATA QUALITY OBJECTIVES

It is the intent of this study to produce data of sufficient quality to be able to meet the following project goals:

- Establish a database of phytoplankton relative abundance and species diversity during algal bloom season in the Central Basin of Puget Sound.
- Investigate relationships between synoptically collected physical and chemical parameters and species presence and relative abundance.
- Provide data of acceptable quality and comparability for internal and external users.

Phytoplankton quality assurance review will comprise several aspects including maintenance of taxonomic keys, photo referencing for verification when necessary, and diversity and abundance analysis by an independent taxonomist. Water quality data will undergo rigorous quality assurance review, which will assess, among other things, accuracy, precision and bias, representativeness, completeness, and comparability. Quality control/quality assurance procedures are provided in Section 6.0.

2.3.1 Precision, Accuracy, and Bias

Precision is the degree of agreement between replicate analyses of a sample under identical conditions and is a measure of the random error associated with the analysis, usually expressed as Relative Percent Difference (RPD) or Relative Standard Deviation (RSD). Accuracy is the measure of the difference between an analytical result and the true value, usually expressed as percent. The accuracy of a result is affected by both systematic errors (bias) and random errors (imprecision). Bias is the systematic or persistent distortion of a measurement process that causes errors in one direction. Precision, accuracy, and bias for water quality data may be evaluated by one or more of the following quality control (QC) procedures:

- Analysis of various laboratory QC samples such as method blanks, matrix spikes, certified reference materials, duplicates and positive and negative controls.
- Collection and analysis of field replicate samples. Field replicate results from lab analysis should exhibit a relative percent difference (RPD) less than 150 percent in order

for the evaluation of the spatial and areal chemical concentrations to be meaningful. Field replicate results for CTD measurements should exhibit RPDs <20%.

- Laboratory QC results will be evaluated against the control limits presented in Appendix A. The acceptance limits defined by the laboratory will meet the needs of this project.

Due to limited resources, no field replicates will be collected to allow estimation of precision. The objective for the phytoplankton data quality is to produce an estimate of accuracy; this must be obtained before a realistic variability target for this type of analysis can be established. Analytical variability will be estimated through independent analysis by a second taxonomist. Because a different taxonomic analytical method is used by the second taxonomist, comparison of the results will reflect both systematic and random errors.

2.3.2 Representativeness

Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point, or an environmental condition. Samples for chemistry and phytoplankton community analyses will be collected from stations with pre-selected coordinates to represent specific site locations and/or fill data gaps. Water chemistry and phytoplankton community analyses will be performed on samples collected simultaneously, to minimize variation in the chemical, biological, and physical composition of the samples. Following the guidelines described for sample collection and sample processing (Section 4) will help ensure that samples are representative. Laboratory representativeness is achieved by proper preservation and storage of samples along with appropriate subsampling and preparation for analysis.

2.3.3 Completeness

Completeness is defined as the total number of samples analyzed for which acceptable analytical data are generated, compared to the total number of samples to be analyzed. Sampling at stations with known position coordinates in favorable conditions, along with adherence to standardized sampling and testing protocols will aid in providing a complete set of data for this project. The goal for completeness is 100 percent. If 100 percent completeness is not achieved, the project manager will evaluate if the data quality objectives can still be met or if additional samples may need to be collected and analyzed.

2.3.4 Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another. For chemistry data, this goal is achieved through using standard techniques to collect and analyze representative samples, along with standardized data validation and reporting procedures. By following the guidance of this SAP, the goal of comparability for water quality data will be achieved. For phytoplankton taxonomy data, comparability is achieved as best possible using external validation and a digital photo library. Comparability goals have not been established for qualitative relative abundance to date because no standard protocols exist. However, King County will explore establishment of standards for this method.

3.0 SAMPLE COLLECTION METHODS AND TECHNIQUES

This section describes sample collection procedures that will be followed to help ensure that project data quality objectives are met. Included in this section are station positioning, sample collection and processing procedures, health and safety requirements and field documentation.

3.1 STATION POSITIONING

Station positioning for Pt. Jefferson (KSBP01) and East Passage (NSEX01) stations will be accomplished using a Global Positioning System (GPS) which will collect geographic coordinate data in real time. Real-time navigational capability is achieved through the use of Nobeltec Admiral software on a shipboard laptop computer; this will be used for samples collected from the *RV Liberty.* The captain will navigate as close as possible to locator coordinates during CTD deployment and sample collection. Proposed station coordinates are shown in Table1.

The Quartermaster Harbor station samples will be collected from the end of the dock where the permanent mooring has been installed.

Table 1. Sampling Station Coordinates

Station Name	LIMS locator	Northing	Easting
East Passage	NSEX01	134701	1255331
Jefferson Point	KSBP01	275214	1247880
Quartermaster Harbor	MSWH01	147976	1236667

Note: Coordinates are in NAD 83

3.2. SAMPLE COLLECTION METHODS

3.2.1 In-situ measurements

A permanent mooring installed at the Quartermaster Harbor station (MSWH01) in 2007 will provide *in situ* measurements of pH, turbidity, dissolved oxygen, salinity, fluorescence (chlorophyll-*a*), and temperature at approximately one meter depth. The meteorological instruments, measuring PAR, wind speed and direction, precipitation and relative percent humidity, are above the water surface. The latter instrument is known as the mooring. The manufacturer's specifications for the sensors can be viewed in Appendix B. Mooring data are collected every 15 minutes and transmitted to a public website for real-time viewing (http://www.ysieconet.com/public/WebUI/Default.aspx?hidCustomerID=165).

A conductivity-temperature-depth (CTD) system will be used for in situ measurements at deep water stations NSEX01 and KSBP01. The standard operating procedure (SOP) "SBE 25 SEALOGGER CTD" #220v3 provides a full description of CTD sampling protocols. A brief summary is provided here. An SBE 25 SEALOGGER CTD, manufactured by SeaBird

Electronics of Bellevue, Washington will be used to profile the water column. In addition to measuring conductivity, temperature, and depth, the system measures dissolved oxygen, chlorophyll-*a* fluorescence, PAR and light transmission (an index of turbidity). Salinity and density are calculated from direct measurements of temperature and conductivity.

The CTD is deployed from the research vessel by hydraulic winch and allowed to equilibrate to surface conditions for approximately five minutes before data recording begins. The CTD is lowered at a specified descent rate from the surface to approximately 25 meters depth (downcast). Data are recorded both on the downcast and during the instrument's return to the surface, the upcast. Data are recorded to a datalogger at a frequency of eight-hertz or approximately eight recordings per second.

Upon retrieval of the instrument, data are uploaded to a laptop computer from the datalogger prior to the next cast. System software converts parameter data into surface-to-depth-to-surface profiles. Temperature, dissolved oxygen, chlorophyll-*a* and salinity are field-reviewed for quality control. The CTD optics are rinsed with reagent water and the instrument is stored aboard the R/V Liberty between sampling stations (King County, Jan., 2003). CTD parameters and associated detection limits can be found in Appendix A.

Data comparability will be assessed after each water column sampling event by comparing CTD plots for each parameter to expected field conditions. Current CTD plots will also be compared with long-term trend analysis for each location and parameter. If any data anomalies are noted, a Data Anomaly Form will be filled out and stored with the sample data. CTD data are also loaded to the CTD database and are available online at http://dnr.metrokc.gov/wlr/waterres/marine/DownloadData.aspx . A "Field Observations" sheet will be completed by the crew for each day to reflect atmospheric conditions, algae blooms, etc.

Secchi depth transparency will be determined at each sampling station using a standard 12-inch diameter Secchi disk. Secchi depths will be recorded to the nearest 0.1 meter. As readings may vary depending upon environmental conditions (e.g., waves and glare) and the individual collecting the measurement, all field crew are trained to collect measurements in a consistent manner.

3.2.2 Discrete Water Samples

At the Quartermaster Harbor station (MSWH01), samples will be collected manually at one meter depth using a Scott bottle. The target depth is set to be same as the depth of the mooring that collects conventionals data. This sampler enables depth grab sampling using a handle and manual bottle trigger. This method is described fully in King County Environmental Lab's SOP "Sampling Methods for Stream and River Water" # 214v3.

Water column samples at Pt. Jefferson (KSBP01) and East Passage (NSEX01) will be collected using Niskin bottles from the CTD. The CTD is programmed to collect samples for the ambient monitoring program on the upcast at 7 depths, typically at 1, 15, 25, 35, 55, 100 meters and 5 meters from the bottom. This method is described in King County Environmental Lab's "Sampling Methods for Marine Offshore Water Column" SOP #212v2. In addition to these depths, the CTD will be programmed to collect Niskin bottle samples at 2.5, 3.5, 5.5, 8 and 10 meters for the phytoplankton community monitoring program. After a station is sampled and while the RV Liberty is piloting to another station, chlorophyll-a fluorescence profiles will be reviewed to determine the depth of the maximum concentration. Sample bottles from the surface (1 m) and the depth closest to the chlorophyll-*a* maximum will be retained for processing

and the remaining surface water samples for the phytoplankton monitoring program will be discarded. These bottles will be placed on ice and sent to the laboratory for final processing and analysis.

3.2.3 Phytoplankton Samples

At the Quartermaster Harbor station (MSWH01), phytoplankton samples will be collected manually at one meter using a Scott bottle following the same method as water chemistry samples. Two 1-liter and one 500 mL plastic containers will be filled for phytoplankton analyses.

At deep water stations, phytoplankton samples will be collected using the CTD in the same manner as the deep water station water chemistry samples. Upon retrieval of the CTD, contents of Niskin sample bottles will be aliquoted to two sample bottles for phytoplankton analysis: one 1-liter and one 500 mL clear plastic container. Bottle labels will include LIMS locator, sample depth and date. Additional volume will be used for water quality analyses. The chlorophyll-*a* maximum depth will be determined following the protocol outlined for surface water samples and the sample bottles nearest this depth and from the surface will be retained. Retained bottles will be placed on ice and transported to the laboratory for final processing and analysis.

A 1 liter field duplicate will be collected at the surface depth interval at each deep water station during every sampling event for domoic acid analysis by National Marine Fisheries Service, National Oceanographic and Atmospheric Administration (NOAA). In addition, one field duplicate per month will be split from the 1 L phytoplankton sample bottle as a phytoplankton QC sample. The location and depth of this duplicate will vary to avoid biasing the QC results.

Table 2. Sample Collection

Station	Locator	Depth	Sample Purpose	LIMS sample #[5.]	Bottle	Volume
Quartermaster Harbor	MSWH01	1 m	Phyto ID - preserved	LXXXXX-a	clear HDPE	1 L
			Phyto ID – live	LXXXXX-a	clear HDPE	500 mL
			Water Quality Chemistry[1.]	LXXXXX-a	(1) clear & (1) amber HDPE	250 ml each
			Phyto ID – QC confirmation[2.]	LXXXXX-b	clear HDPE	500 mL
			Secchi reading	LXXXXX-a[4.]	none	NA
Point Jefferson	KSBP01	1 m	Phyto ID - preserved	LXXXXY-c	clear HDPE	1 L
			Phyto ID – live	LXXXXY-c	clear HDPE	500 mL
			Water Quality Chemistry[1.]	LXXXXY-c	(1) clear & (1) amber HDPE	250 ml each
			Domoic Acid[3.]	LXXXXY-c	clear HDPE	1 L
			Secchi, CTD and LiCor readings	LXXXXY-c[4.]	none	NA
		Chl *a* max	Phyto ID - preserved	LXXXXY-d	clear HDPE	1 L
			Phyto ID – live	LXXXXY-d	clear HDPE	500 mL
			Water Quality Chemistry[1.]	LXXXXY-d	(1) clear & (1) amber HDPE	250 ml each
			Phyto ID – QC confirmation[2.]	LXXXXY-e	clear HDPE	500 mL
East Passage	NSEX01	1 m	Phyto ID - preserved	LXXXXX-f	clear HDPE	1 L
			Phyto ID – live	LXXXXX-f	clear HDPE	500 mL

Station	Locator	Depth	Sample Purpose	LIMS sample #[5.]	Bottle	Volume
			Water Quality Chemistry[1].	LXXXXX-f	(1) clear & (1) amber HDPE	250 ml each
			Domoic Acid[3].	LXXXXX-f	clear HDPE	1 L
			Secchi, CTD and LiCor readings	LXXXXX-f[4].	none	NA
		Chl *a* max	Phyto ID - preserved	LXXXXX-g	clear HDPE	1 L
			Phyto ID – live	LXXXXX-g	clear HDPE	500 mL
			Water Quality Chemistry[1.]	LXXXXX-g	(1) clear & (1) amber HDPE	250 ml each
			Phyto ID – QC confirmation[2.]	LXXXXX-h	clear HDPE	500 mL

Notes:

1. This table lists bottles and measurements routinely collected during the first week of each month during which samples are collected. Samples collected during the third week of each month will be collected in conjunction with routine Marine Ambient Offshore Monitoring samples and will include additional Water Quality Chemistry bottles.

2. Phyto ID – QC confirmation sample subcontracted, collection location will rotate. Samples are preserved with Lugol's Solution. 2008 schedule: KSBP01 @ chl *a* max depth: May, August

NSEX01 @ chl *a* max depth: June, September

MSW01 @ 1 m depth: July

3. Domoic Acid samples filtered and frozen, pending analysis by NOAA / NMFS. Samples will be prelogged for the DOMOIC-ACID product. Results will not be entered into LIMS

4. Secchi readings will have the same LIMS sample number as the associated Phyto ID bottle.

5. The "LIMS sample numbers" in the table represent the scheme that will be used for samples collected during the third week of each month. Samples collected during the first week of each month will have an assigned LIMS "L number" that will be unique to the samples collected for this study. Samples collected during the third week of each month will have the same "LIMS sample number" (e.g., LXXXXX-a in the table above) as the Ambient Offshore Monitoring Stations they are collected with for all aliquots with 2 exceptions. The Phyto ID – QC confirmation samples are considered separate samples and will have a unique sample number, although they will share the same "L number" (e.g., LXXXXX in the table above). The Quartermaster Harbor samples are typically collected with Intertidal stations due to their shallow depth. However, they are considered to represent Offshore waters, and will be added on to the L number corresponding to the southern end of the Offshore run.

NA – Not applicable.

Phyto ID –Phytoplankton identification and qualitative abundance

3.2.4 Sample Storage and Delivery

All sample containers will be stored in an insulated cooler on ice immediately after collection to maintain them at a temperature of approximately 4° C until delivery to the laboratory. At the end of each sampling day, all samples will be transported to the King County Environmental Laboratory (KCEL). The sample delivery person will relinquish all samples to the sample login person upon arrival at the KCEL. The date and time of sample delivery will be recorded, and both parties will then sign off in the appropriate sections on the Laboratory Work Order/Chain of Custody form at this time. Once completed, the original will be archived in the respective project file. Samples delivered after regular business hours will be stored in a secure refrigerator until the next day.

3.3 CHAIN-OF-CUSTODY PROCEDURES

Field chain-of-custody procedures will be followed from the time a sample is collected until it is relinquished to the analytical laboratory. Laboratory supplied field sheets with appropriate signatures and dates will be used to document chain-of-custody in the field. Separate forms will be used for the domoic acid samples because their destination is the NOAA laboratory not KCEL. Information to be included on the fieldsheet will include sample number, date of sampling, names of all sampling personnel, and requested analyses.

A sample will be considered “in custody” when in the possession of sampling personnel or in a secured sampling area such as onboard the research vessel. Samples will not be considered in custody when left unattended onboard the vessel while docked or in an unlocked field vehicle. Custody seals will be placed on the sample cooler when it is not in the custody of a member of the sampling team.

Chain-of-custody will be maintained throughout the analytical phase of the project according to standard KCEL protocols and any subcontracting laboratory standard operating protocols.

3.4 SAMPLE PROCESSING

Surface water samples will be processed for chemistry and taxonomic analysis using the methods described herein.

3.4.1 Chemistry Testing

Ammonia, nitrate/nitrite, total suspended solids, silica, total phosphorus, phaeophytin, and chlorophyll-*a* will be consistently collected. A “CTD QC” sample set is collected at each station at one depth which includes laboratory samples for salinity and Winkler dissolved oxygen. One field replicate for every 20 samples (with a minimum of one field replicate per day) is also collected. Samples will be stored in ice-filled coolers until delivery to the laboratory.

3.4.2 Phytoplankton Sample Processing and Preservation

Final processing of the phytoplankton samples will be completed by KCEL Aquatic Toxicology Unit (KCEL SOP #439v). At KCEL, the samples will immediately be transferred to a dark cooler.

In the lab, the 1 L samples collected for phytoplankton preservation will be transferred to a conical container and buffered formalin will be added (final concentration 0.4% v/v) no later than 24 h after arrival. No preservative will be added to the 500 mL sample. The samples will be allowed to settle at room temperature for at least 24 h. The overlying water will be carefully removed and the concentrated sample carefully transferred to a scintillation vial. Vials will be kept in a cool dark place and saved for archiving.

The duplicate sample for QC will be mixed gently and transferred to a 125 mL amber plastic container. Acidified Lugol's iodine (0.2 mL) will be added, swirled, and additional sample volume will be added to eliminate any headspace. The sample will be packed and shipped at room temperature within a week of collection.

The 500 mL sample will be concentrated by reverse filtration using a 5 µm nylon mesh and saved in a lab refrigerator for up to 4 days for live identification.

The 1L sample for domoic acid analysis by NOAA will be will be processed by KCEL within 24 hours of collection. Samples will be relinquished to NOAA for analysis upon their request, based on their own domoic acid monitoring program. Processing by KCEL will involve filtering the sample onto a Millipore HA filter using a pump and filter system. The sample will then be mixed gently by repeated inversion, the volume measured, and filtered at low pressure. The filter will then be removed with forceps, folded in half, packed in aluminum foil and stored at -20º C until delivery to NOAA.

4.0 SAMPLE ANALYSIS

The analytical methods to be used for water quality and phytoplankton analyses are summarized in this section. Specific data qualifiers used for water quality data are defined herein.

4.1 WATER QUALITY PARAMETERS

Ammonia, nitrate/nitrite, total suspended solids, silica, total phosphorus, phaeophytin, and chlorophyll-*a* will be routinely collected and analyzed. The following section provides a brief description of each of the methods for conventional analyses. Analytical method references and associated detection limits are summarized in Appendix A.

- Ammonia Nitrogen – This is an automated analysis of the ammonium ion. When ammonium is introduced to a basic medium it forms ammonia gas. In the membrane module, the ammonia gas diffuses across a polypropylene membrane and is retained in a slightly acidic stream. Phenol and alkaline hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroferricyanide. The absorbance is measured at 660 nanometers (SM4500-NH3-G).

- Nitrate+Nitrite Nitrogen – This is an automated analysis where nitrate is converted to nitrite by cadmium reduction. Nitrite is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)- ethylenediamine dihydrochloride to form a highly colored azo dye, which is proportional to the nitrite concentration. Nitrite alone may be determined by omitting the reduction step. The absorbance is measured at 540 nm (SM4500-NO3-F).

- Total Phosphorus – Most forms of phosphorus are oxidized to orthophosphate using potassium persulfate in an acidic medium and high temperature. The digestate is analyzed by automated colorimetry for orthophosphate. Orthophosphate reacts with molybdenum VI and antimony III in an acidic medium to form an antimonyphosphomolybdate complex. This complex is subsequently reduced with ascorbic acid to form a blue color and the absorbance is read at 660 nm (SM4500-P-B, F).

- Silica – This is an automated analysis of silica. Silica in solution as silicic acid or silicate reacts with molybdate reagent in acid media to form β-molybdo-silicic acid. The complex is reduced by ascorbic acid to form molybdenum blue. The absorbance is measured at 660 nm (WHITLEDGE 1981).

- Chlorophyll-*a* and phaeophytin-*a* – Sample is concentrated by filtering a measured volume of sample through a glass fiber filter at low vacuum. Pigment is extracted from the phytoplankton by ultrasonic disruption of the cells in an acetone medium. Concentration of chlorophyll-*a* is determined fluorometrically. Because phaeophytin-a is a positive interferent, chlorophyll-*a* is converted to phaeophytin-a by acidification and then the concentration of phaeophytin-*a* is determined, allowing calculation of the original concentrations of these related pigments. The fluorometric method provides a procedure for determination of low level chlorophyll-*a* and phaeophytin-*a*, in marine phytoplankton using fluorescence detection (EPA 445.0).

- Total Suspended Solids – A well-mixed sample is filtered through a preweighed glass fiber filter. The residue retained on the filter is dried to constant weight at 103 to 105°C. The increase in weight of the filter represents the total suspended solids (SM2540-D).

- Dissolved Oxygen – Dissolved oxygen (DO) by the Winkler titration, Azide modification method. The sample is treated with manganous sulfate followed by Alkali-Iodide-Azide (A-I-A) solution immediately after sample collection. Dissolved oxygen rapidly oxidizes an equivalent amount of the dispersed divalent manganous hydroxide to form a brown precipitate, Mn(OH)2. Upon acidification with sulfuric acid, manganic hydroxide forms manganic sulfate, which then acts as an oxidizing agent to liberate free Iodine from the alkali-iodide. The iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample, is then titrated with a standard solution of sodium thiosulfate. The titration end point is determined visually with a starch indicator (SM4500-O-C).

- Salinity – Conductance of an electrical current through the sample is measured and compared to the conductivity of a standard seawater solution. This is an analysis of salinity in saline waters, between 3-42 on the Practical Salinity Scale (SM2520-B).

- Turbidity – The method is based on a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered

light, the higher the turbidity. The range of turbidity from 0 to 2500 nephelometric units (NTU), higher values may be obtained with dilution of the sample (SM2130-B).

4.1.1 Data Qualifiers

The data qualification flags, which will be used by the KCEL for this project, are presented in Table 3. These data qualifiers address situations that require qualification and generally conform to QA1 guidance (Ecology, 1989a). The KCEL qualifiers indicating <MDL and <RDL have been used as replacements for the *T* and *U* qualifier flags specified under QA1 guidance. These qualifiers may be updated in the future to reflect changes in Washington Department of Ecology guidelines.

Table 3. Laboratory Data Qualifiers

Qualifier	Description
General	
H	Indicates that a sample handling criterion was not met in some manner prior to analysis. The sample may have been compromised during the sampling procedure or may not comply with holding times, storage conditions, or preservation requirements. The qualifier will be applied to applicable analyses for a sample.
R	Indicates that the data are judged unusable by the data reviewer. The qualifier is applied based on the professional judgment of the data reviewer rather than any specific set of QC parameters and is applied when the reviewer feels that the data may not or will not provide any useful information to the data user. This qualifier may or may not be analyte-specific.
<MDL	Applied when a target analyte is not detected or detected at a concentration less than the associated method detection limit (MDL). MDL is defined as the lowest concentration at which an analyte can be detected. The MDL is the lowest concentration at which a sample result will be reported.
<RDL	Applied when a target analyte is detected at a concentration greater than or equal to the associated MDL but less than the associated reporting detection limit (RDL). RDL is defined as the lowest concentration at which an analyte can reliably be quantified. The RDL represents the minimum concentration at which method performance becomes quantitative and is not subject to the degree of variation observed at concentrations between the MDL and RDL.
RDL	Applied when a target analyte is detected at a concentration that, in the raw data is equal to the RDL.
TA	Applied to a sample result when additional narrative information is available in the text field. The additional information may help to qualify the sample result but is not necessarily covered by any of the standard qualifiers.

4.2 DOMOIC ACID ANALYSIS

As soon as possible after arrival at KCEL (within 24 hours) cells will be collected and frozen for domoic acid analysis, a biotoxin produced by certain species of Pseudonitzschia. Cells will be collected on 0.45 µm Millipore HA filters from 1 liter of sample using vacuum at low pressure. The filter will then be folded in half, packed in aluminum foil, and stored at -20°C. If an increase in *Pseudonitzschia* abundance is observed at that station and/or upon request, the filter will be handed over to the National Marine Fisheries Service Marine Biotoxin Program at NOAA for analysis of domoic acid.

4.3 PHYTOPLANKTON ANALYSIS

Within the first three days, the unpreserved, concentrated sample will be examined by microscope for species identification, focusing on motile taxa and taxa that degrade under formalin preservative (primarily naked dinoflagellates and the bloom-forming *Heterosigma*). A one-tenth mL of concentrate (or volume indicated by chamber manufacturer) will be placed on a Palmer-Maloney style chamber with cover slip. Objectives of 10 – 40x or 100x magnification will be used on a Nikon 50i Microscope with differential interference contrast (DIC) and phase-contrast capabilities.

Preserved samples will be examined for taxonomy within 3 months of collection. All unique taxa will be identified and recorded in each sample. Phytoplankton will be identified to the lowest practical level. The qualitative abundance analysis will identify genera as dominant, subdominant, or present. Dominant genera will be assigned when a genus is judged to be present by count in >50% of the subsample. Subdominant will be assigned when a genus is judged to be less than 50% or greater than 25% of the subsample. By default, all other taxa will be assigned Present. These procedures will be performed using the same method described for the unpreserved sample. This phytoplankton analytical method selected was chosen because it is efficient and effective for qualitative abundance analysis. Also, the equipment is readily available at KCEL.

A semi-quantitative protocol will be tested to define abundance criteria with the intention of establishing greater interanalyst comparability and intersource comparability for the King County relative abundance data. Using a gridded slide, coverslip or eyepiece, relative abundance will be estimated based on the following criteria:

1. Tally a set number of parcels within the grid for dominance (e.g. 9 parcels), using cell number as the criterion. Record the dominant genus for each parcel.

2. For each genus, count the number of parcels where it was dominant.

3. Determine overall dominance as dominant in >50% of parcels, subdominance as dominant in <50% of parcels.

If this or some other protocol can be established that defines relative abundance categories more discretely than professional judgment, it will be adopted and shared with other organizations (e.g. NOAA) that examine phytoplankton for Harmful Algal Blooms (HAB) or other programs. The benefits include not only quality assurance internally but also increasing comparability and usability of the King County data. HAB researchers contacted during study design of the King County program either conduct quantitative analysis or rely solely on

professional judgment in qualitative analysis. Some expressed interest in adopting qualitative standards if they are established.

4.3.1 Photographic Imaging

The KCEL is purchasing a Nikon DSU2 digital camera microscope system with the Nikon Elements B Software and Database Module for image acquisition and storage. Phytoplankton photographs will be stored digitally for two main purposes: to share with a local expert when identification assistance is needed, and to create an image library as a reference guide.

5.0 ANALYTICAL QA/QC PROCEDURES

5.1 WATER CHEMISTRY SAMPLES

Quality control samples will be analyzed at a frequency of one per analytical batch or a minimum of one per 20 analytical samples. A comprehensive list of laboratory QC samples for conventional analyses and associated control limits are listed in Appendix A.

5.2 PHYTOPLANKTON SAMPLES

Quality control of phytoplankton analyses for taxonomic identification and qualitative abundance will be limited due to the absence of field replicates and due to limited analytical resources which prohibit multiple subsample analyses. The analyst will conduct taxonomic identification of subsamples in different states: live subsample, preserved subsample and a subsample of preserved concentrate for relative abundance categorization. In addition, uncertainties in taxonomic identification will be confirmed via photo transmittal to phytoplankton expert, Dr. Rita Horner of the University of Washington.

Quality assurance of taxonomic identification will involve independent external taxonomic identification of a duplicate of 10% of samples or one sample per 10 collected. The QA taxonomist will be Nicky Haigh of Nixy Consulting and her qualifications can be found in Appendix C. Sample preparation will consist of concentration using the same KCEL method described for all other samples (Section 4.3). Samples shipped for QA taxonomy will be preserved with acidified Lugol's solution as preferred by the taxonomist[1]. Nixy Consulting will use a slightly different method for identifying phytoplankton. For identification, a 1 mL aliquot of concentrated sample will be examined in a Sedgewick Rafter Slide at 100X magnification using a Leitz Laborlux D compound microscope, and phytoplankton species will be identified to the lowest practicable level. Until King County develops a final qualitative method, these species will be qualitatively assessed as either "dominant", "subdominant", or "present" using best professional judgment.

QA sample results will be directly compared to the associated split sample results from KCEL. If discrepancies exist at genus level or below between KCEL and Nixy Consulting identification results, the possible sources of the variability will be examined on a case-by-case basis. The

[1] The QA taxonomist has chemical sensitivity to formalin and regularly uses Lugol's similar to other phytoplankton taxonomists. Formalin performs more or less as well as other preservatives and is the best for sample archiving. King County chose formalin as a preservative because of its long term storage benefits.

focus will be to differentiate systematic taxonomic differences from random error. Systematic variability may stem from differences in preservative, analytical method, taxonomist experience or some other source. However, differences due to preservative or analytical method are predicted to be insignificant, expected to present themselves systematically and should be easy to detect. Overall, the independent QA analysis has the primary purpose of providing data for estimation of variability between analysts.

Once the microscope with camera and database software is purchased and installed, photographs of specimens will be taken from select samples to build an image library. The images will function as an archive of algal taxa collected from Puget Sound.

6.0 SAMPLE DOCUMENTATION

This section provides guidance for documenting sampling and data gathering activities. The documentation of field activities provides important project information that can support data generated by laboratory analyses.

6.1 SAMPLE NUMBERS AND LABELS

Unique sample numbers will be assigned to each sampling location. Sample numbers will be assigned prior to the sampling event and waterproof labels generated for each sample container.

6.2 FIELD NOTES

Field notes will be maintained for all field activities, both the collection of samples and the gathering of environmental data. Field notes will be recorded in blue or black ink on pre-printed field sheets, prepared specifically for this project. A sample field sheet is shown in Figure 2. Information recorded on field notes will include, but not be limited to:

- initials of field personnel,
- sample number,
- sample station locator information,
- sample depth,
- date of sample collection,
- weather conditions

Additional information that may be recorded on the field sheets includes sampling methodology, time of sample collection, and any deviations from established sampling protocols. Additional anecdotal information pertaining to observations of unusual sampling events or circumstances may also be recorded on the field sheets.

6.3 FIELD ANALYTICAL RESULTS

Field analytical results will be recorded on field sheets in a manner that easily identifies the information as analytical data. All entries will be recorded in waterproof, blue or black ink.

Figure 2. Field Sheet (Example)

Fieldsheet ID: 421235_22JUN1999_101133 Page: 1

MAJOR LAKES (wtr col)

Project Number: 421235 Personnel: _____

Sample Number	P15790-1	P15790-2	P15790-3
Locator	0618	0623	0625
Short Loc. Desc.		Rosemnt SD	Sammslough
Locator Desc.		LAKE SAMM/WEST SHORE-ROSEMONT STOR	Lake Sammamish
Site	MAJOR LAKES	MAJOR LAKES	MAJOR LAKES
Sample Depth			
Collect Date			
Comments			
EH, FIELD			
SED DEPTH			
SED SAMP RANGE			
SED TYPE			
TIME			
Dept., Matrix, Prod			
	3 FRSHWTRSED AVS	3 FRSHWTRSED AVS	3 FRSHWTRSED AVS
	3 FRSHWTRSED NH3	3 FRSHWTRSED NH3	3 FRSHWTRSED NH3
	3 FRSHWTRSED PSD	3 FRSHWTRSED PSD	3 FRSHWTRSED PSD
	3 FRSHWTRSED TOC	3 FRSHWTRSED TOC	3 FRSHWTRSED TOC
	3 FRSHWTRSED TOTP	3 FRSHWTRSED TOTP	3 FRSHWTRSED TOTP
	3 FRSHWTRSED TOTS	3 FRSHWTRSED TOTS	3 FRSHWTRSED TOTS
	3 FRSHWTRSED TOTSULFIDE	3 FRSHWTRSED TOTSULFIDE	3 FRSHWTRSED TOTSULFIDE
	6 FRSHWTRSED HG-CVAA	6 FRSHWTRSED HG-CVAA	6 FRSHWTRSED HG-CVAA
	6 FRSHWTRSED PP ICPMS	6 FRSHWTRSED PP ICPMS	6 FRSHWTRSED PP ICPMS
	7 FRSHWTRSED BNA	7 FRSHWTRSED BNA	7 FRSHWTRSED BNA
	7 FRSHWTRSED CHLOROBENZENES	7 FRSHWTRSED CHLOROBENZENES	7 FRSHWTRSED CHLOROBENZENES
	7 FRSHWTRSED CLPESTPCB	7 FRSHWTRSED CLPESTPCB	7 FRSHWTRSED CLPESTPCB
	7 FRSHWTRSED HERB	7 FRSHWTRSED HERB	7 FRSHWTRSED HERB
	7 FRSHWTRSED OPPEST	7 FRSHWTRSED OPPEST	7 FRSHWTRSED OPPEST
	7 FRSHWTRSED TRIBUTYLTIN	7 FRSHWTRSED TRIBUTYLTIN	7 FRSHWTRSED TRIBUTYLTIN
	7 FRSHWTRSED WTPH-HCID	7 FRSHWTRSED WTPH-HCID	7 FRSHWTRSED WTPH-HCID

End of Fieldsheet.

Consistent sample handling procedures are necessary to maintain sample integrity and provide high-quality defensible data. This section provides requirements for proper sample containers, labeling, preservation and storage, and chain-of-custody.

7.0 DATA MANAGEMENT

Water quality and phytoplankton data will be entered by KCEL staff into the Laboratory Information Management System (LIMS), KCEL's analytical database. Phytoplankton data will be transferred to the Data Manager in Excel format for uploading into a SQL server-based database customized with an Access data entry interface. This database will house marine and freshwater phytoplankton analytical data from King County's monitoring programs. Synoptic water quality data will be imported on a regular basis. The database will link to the Integrated Taxonomic Information System (ITIS, www.itis.gov), which will enable download of upper taxonomic nomenclature and assign a numeric code. The coding system assists with tracking of nomenclature changes, allowing quick and thorough updating of taxonomic names in the phytoplankton database.

The phytoplankton database will provide query and reporting features to aid in data review and data analysis. Editing and downloading capabilities will be included.

8.0 DATA ANALYSIS, RECORD KEEPING, AND REPORTING

The KCEL will provide a 30-day turnaround time for all water sample analytical data, and a 90-day turnaround time for phytoplankton data based upon login date of sample. Contract QA phytoplankton data will be provided within 30 days of receipt of sample. Each KCEL laboratory unit will provide a Data Anomaly Form (DAF) describing analytical anomalies if any occurred. All data received from subcontracted laboratories will be reported to the Project Data Manager in an Excel template.

8.1 INTERPRETATION OF DATA

Phytoplankton data and synoptically-collected water quality data will be reviewed by Science and Technical Support staff on an annual basis at a minimum. Data will be reported annually as part of the Marine Water Quality Status Report. In addition, interpretive analysis will be conducted every five years and reported as part of the Marine Water Quality Status Report.

8.2 RECORD KEEPING

All field analysis and sampling records, custody documents, raw lab data, data summaries, and case narratives will be archived according to KCEL policy.

Concentrated, formalin-preserved phytoplankton samples will be archived for two years or until a digital image library is developed. No samples will be disposed of without consent of the Project Manager.

8.3 REPORTING

Project data will be presented to the project and data managers in a format that will include the following:

- spreadsheets of all chemistry data in electronic format (provided by the KCEL);
- a narrative of chemistry data anomalies, if any, including supporting QC documentation (provided by the KCEL); and
- phytoplankton data in Excel format using the template provided in Appendix D.

Domoic acid analytical data will not be delivered to King County and the QA taxonomists results will be delivered directly to the Data Manager in the template provided in Appendix E.

9.0 PROJECT MANAGEMENT

Project team members and their responsibilities are summarized in Table 4.

Table 4. Project Team Members and Responsibilities

Name/Telephone	Title	Affiliation	Responsibility
Katherine Bourbonais 206- 684-2382	Laboratory Project Manager	KC Environmental Laboratory	Coordination of analytical activities, lab QA/QC, and data reporting.
Jenée Colton 206-296-1970	Toxics and Contaminant Assessment Group Scientist	KC Science and Technical Support	SAP preparation, study design support and data management
Colin Elliott 206-684-2343	Quality Assurance Officer	KC Environmental Laboratory	Overall lab QA/QC
Nicky Haigh	Taxonomist/ Owner	Nixy Consulting	External QA taxonomy
Gabriela Hannach 206-684-2301	Environmental Laboratory Scientist	KC Environmental Laboratory	Method development, phytoplankton ID and abundance
Jean Power 206-684-2393	Environmental Laboratory Scientist	KC Environmental Laboratory	Coordination of sampling activities, field QA/QC, and field analyses.
Kimberle Stark 206-296-8224	Marine Group Lead Scientist	KC Science and Technical Support	Project manager for marine phytoplankton and other marine monitoring.

KC = King County

10.0 HEALTH AND SAFETY REQUIREMENTS

The following general health and safety guidelines have been provided in lieu of a site-specific Health and Safety Plan. These guidelines will be read and understood by all members of the sampling crew prior to any sampling activities.

- All crew of the research vessel will have received annual vessel safety training that will include proper chain of communication, equipment operation, and safe boating practices.
- Sampling personnel will wear chemical-resistant gloves whenever coming into contact with any potentially hazardous, caustic, or biologically unsafe substances.
- No eating, drinking, smoking, or tobacco chewing by sampling personnel will be allowed during active sampling operations.
- All sampling operations will be conducted during daylight hours.
- All accidents, "near misses," and symptoms of possible exposure will be reported to a sampler's supervisor within 24 hours of occurrence.
- All crewmembers will be aware of the potential hazards associated with chemicals used during the sampling effort. Appropriate gloves and eye protection will be worn when reagents are being added to Winkler DO samples and for the addition of Lugol's, if done in the field.

Several hazards are inherent to offshore water column sampling. General vessel safety, physical hazards unique to water sampling, and chemical hazards are discussed in sections 6.1.1 through 6.1.3.

10.1 SAFETY AND HAZARDOUS MATERIALS MANAGEMENT

The 43-foot long research vessel *Liberty* typically requires three crewmembers to conduct routine water sampling operations. All sampling personnel will follow standard safety procedures while on board the sampling vessel. The *Liberty* skipper has ultimate responsibility for safety while the vessel is underway. During deployment and retrieval of the CTD, the boom operator has ultimate responsibility for safety on the rear deck. Sampling personnel are required to wear steel-toed boots and hard hats during deployment and retrieval of the CTD. Other recommended safety equipment includes foul-weather gear, eye and ear protection on the back deck while the boat is cruising at high speeds. To avoid injury, loss of equipment and possible loss of life, strict communication procedures between all crewmembers **must** be followed. A "hands free," 2-way loudspeaker system from cabin to rear deck is provided and should be used appropriately. The *Liberty* is also equipped with a video camera which allows the boat skipper to observe operations on the rear deck.

10.2 BOATING SAFETY

To help prevent accidents and ensure adequate preparation for emergencies that may possibly arise, the following safety equipment will be required on the KC research vessel *Liberty*:

- one personal flotation device for each crew member, as well as at least one throwable flotation device
- an accessible, clearly labeled, fully stocked first-aid/CPR kit
- an accessible and clearly labeled eye wash
- one (preferably two) VHF marine radio(s) with weather channel
- a cellular telephone
- a horn
- navigation lights
- an emergency life raft with oars or paddles;
- an anchor and suitable line
- signal flares
- a reach pole or shepherd's hook.

Personal protective equipment will be selected and used that will protect workers involved in crane/CTD sampling from the hazards and potential hazards likely to be encountered. Minimum required personal protective equipment for marine water column sampling shall include the following:

- hard hat;
- steel-toe rubber boots;
- chemical-resistant gloves (Nitrile, vinyl, or latex);
- Appropriate personal anti-exposure clothing or gear.

Recommended additional personal protective equipment will include rain gear and hearing protection when cruising at high speeds, and eye protection for use against exposure to sunlight and glare or any hazardous chemicals. It is also suggested that crewmembers minimize their skin exposure to the sun, either by wearing protective clothing or by using sunscreen (which is kept onboard the *Liberty*).

10.3 OPEN WATER HAZARDS

There are many hazards associated with operating a research vessel. The majority of the hazards are associated with weather, equipment failure, and inability to stay on board. Prior to embarking on a sampling run, local weather forecasts should be studied. These data should provide the skipper with the ability to determine if weather conditions are acceptable or if they are predicted to change. High winds are typically the main determining factor. A skipper should have knowledge of what wind ranges are acceptable for the particular sampling vessel. Other weather factors may include fog or currents, which are generally acceptable but could contribute to unsafe conditions. Vessels should be in a consistent maintenance program and the skipper should have a good knowledge of all vessel systems in case of failure. Vessels are to have an excess of the expected fuel required to complete the sampling run. Prior to entering shipping lanes, the skipper will contact Seattle Traffic for clearance.

11.0 REFERENCES

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King County. 2001. Small Streams Toxicity Study 2001 Sampling and Analysis Plan Addendum. http://dnrlab-web/saps/current/swamp/StreamToxSAPAddendum2001B.doc

PSEP. 1996. Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound. Prepared for U.S. Environmental Protection Agency Region 10, Office of Puget Sound, Seattle, WA and Puget Sound Water Quality Authority, Olympia, WA by King County Environmental Laboratory. Seattle, WA.

Standard Methods for the Examination of Water and Wastewater, 19th ed., 1995. American Public Health Association, Washington, D.C.

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APPENDICES

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APPENDIX A. FIELD INSTRUMENT AND ANALYTICAL QC LIMITS

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CONVENTIONALS ANALYSIS

Table A-1. Conventionals Target Analytes and Detection Limits for Water Samples

Analyte	Method	Units	MDL	RDL
Ammonia Nitrogen	SM 4500-NH3-G	mg/L	0.01	0.02
Chlorophyll-*a*	EPA 445.0 (fluorometric)	ug/L	0.05	0.1
Phaeophyti- *a*	EPA 445.0 (fluorometric)	ug/L	0.1	0.2
Nitrate+Nitrite, Nitrogen	SM 4500-NO3-F	mg/L	0.02	0.04
Silica, Dissolved	Whitledge, 1981	mg/L	0.05	0.1
Total Phosphorus	SM 4500-P-B, F	mg/L	0.005	0.01
Total Suspended Solids	SM 2540-D	mg/L	0.5	10

Table A-2. Conventionals Frequency and Acceptance Criteria for Water Samples

	Method Blank	Lab Duplicate (RPD)	Spike Blank (% Recovery)	Matrix Spike (% Recovery)	LCS (% Recovery)	Check Standard (% Recovery)
Frequency	**1 per batch***	**1 per batch***	**1 per batch***	**1 per batch***	**1 per batch***	**1 per batch***
Analyte						
Ammonia Nitrogen	<MDL	20%	80-120%	75-125%	85-115%	N/A
Chlorophyll-*a*	<MDL	25%	N/A	N/A	N/A	90-110%
Phaeophytin-*a*	<MDL	50%	N/A	N/A	N/A	N/A
Nitrate+Nitrite Nitrogen	<MDL	20%	80-120%	75-125%	85-115%	N/A
Silica, Dissolved	<MDL	20%	80-120%	65-120%	85-115%	N/A
Total Phosphorus	<MDL	20%	80-120%	75-125%	85-115%	N/A
Total Suspended Solids	<MDL	25%	N/A	N/A	80-120%	N/A

* batch = 20 samples or less prepared as a set

< MDL = less than the Method Detection Limit.

RPD = Relative Percent Difference

LCS = Lab Control Sample

N/A = Not Applicable

CTD INSTRUMENTS

REPLICATE MEASUREMENTS

Replicate sensor readings will be reported to LIMS once per cruise. Both the original and replicate samples are true samples and loaded to LIMS. In order to calculate RPD values, QC replicate samples are created in LIMS using the FREP locator and linked back to the original sample. See the table below for acceptable limits.

Table A-3. Conventionals Acceptance Limits

Parameter	DO, Field	Sal, Field	Samp Depth	Sample Temp	Light Transmission, Field
Acceptance Limits	20 % RPD	0.3 PSS	1 or 4 meters	0.3 deg C	20 % RPD

SPLIT SAMPLES AND FIELD REPLICATES

For marine projects, salinity and dissolved oxygen measurements will be analyzed by the KCEL Conventionals Unit using water samples collected synoptically. The frequency will be a minimum of 1 per cast to facilitate the dissolved oxygen post-cruise calibration. The acceptance range for field dissolved oxygen versus Winkler DO is 20% RPD. For salinity, the acceptance range is 0.3 PSS. Field replicates are analyzed at a minimum frequency of 1 per 20 samples or once per day, whichever is more frequent.

METHOD BLANKS

Method Blanks are not appropriate for CTD analysis.

CONTINUING CALIBRATION VERIFICATION

CCV's are not appropriate for CTD analysis.

DEPTH COMPARISON

Following the Bottle Summary calculations, each bottle trip will be compared with the intended sample depths. For samples collected at less than 100 meters, the intended depth and the CTD depth should be within 1 meter. For depths greater than 100 meters, the difference should be no greater than 4 meters.

Table A-4. Detection limits for CTD parameters

Parameter	Units	MDL	RDL	Significant Figures MDL	Significant Figures RDL
Sample Depth	m	n/a	n/a	2	3
Sample Temperature, Field	Deg. C	n/a	n/a	*	*
Dissolved Oxygen, Field	mg/L	0.5	1.0	2	3
Salinity, Field	PSS	n/a	n/a	5	5
Density, Field	kg/m^3	n/a	n/a	7	7
Chlorophyll	ug/L	0.06	0.12	2	3
Light Transmission	% light	0.01	0.01	2	3
Light Intensity (PAR), Field	umol/s m^2	n/a	n/a	**	n/a

*= Round to 0.001

** = Round to 0.1

APPENDIX B. YSI MOORING SPECIFICATIONS

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NEW

YSI 6600 V2 Sonde

With 2 or 4 optical ports and new sensor options

Make the most of your environmental monitoring efforts: The 6600 V2 sonde offers the most comprehensive water quality monitoring package available with simultaneous measurement of conductivity (salinity), temperature, depth or level, pH/ORP. The 6600 V2-4 also measures these parameters: dissolved oxygen, turbidity, chlorophyll, and blue-green algae; the V2-2 measures two of the four parameters simultaneously. Additional calculated parameters include total dissolved solids, resistivity, and specific conductance.

Take advantage of YSI's new optical sensor design and anti-fouling wiper control for improved reliability during extended deployments.

- **Self-cleaning optical sensors with integrated wipers remove biofouling and maintain high data accuracy**
- **Field-replaceable sensors make trips to the field quick**
- **Optimal power management and built-in battery compartment extends *in situ* monitoring periods**

Take Advantage of YSI's New Optical Sensors

In addition to turbidity, chlorophyll, and rhodamine, YSI now offers these optical sensors:

ROX Reliable Optical Dissolved Oxygen

ROX

The ROX sensor uses lifetime luminescence detection technology to offer the most reliable oxygen sensor with the lowest possible maintenance effort. The sensor is insensitive to hydrogen sulfide interference and does not require regular membrane changes.

Blue-Green Algae (BGA)

YSI's fluorescence-based blue-green algae sensors will allow you to monitor blue-green algae populations where their presence is a concern. Whether providing an early warning to an algal bloom, tracking taste and odor-causing species in drinking water supplies, or conducting ecosystem research, YSI BGA sensors will provide sensitive and reliable *in situ* data.

Complete Data Record

The YSI 6600 V2-4 Sonde, with 4 optical sensor ports, is the only instrument available to simultaneously measure dissolved oxygen, turbidity, chlorophyll, and blue-green algae!

Pure Data for a Healthy Planet.®

Upgraded sondes for rugged long-term deployment

*Sensor performance verified**

The 6600 V2 sonde uses sensor technology that was verified through the US EPA's Environmental Technology Verification Program (ETV). For information on which sensors were performance-verified, turn this sheet over and look for the ETV logo.

ETV

6600 Upgrades Available

YSI is committed to offering our customers reliable and cost-effective water monitoring solutions. To this end, we are offering V2 Upgrades for existing 6600s. Upgrades will be available from YSI Authorized Service Centers and will include the new 6600 V2 bulkhead, a ROX Optical Dissolved Oxygen Sensor, and firmware/software upgrades. In addition, the sonde will be fully tested and calibrated by an experienced YSI service technician.

www.ysi.com/v2

To order, or for more info, contact YSI Environmental.

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ISO 9001
ISO 14001
Yellow Springs, Ohio Facility

* Sensors with listed with ETV logo were submitted to the ETV program on the YSI 6600 EDS. Information on performance characteristics of YSI water quality sensors can be found at www.epa.gov/etv or call YSI at 800/897-4151 for the ETV verification report. Use of ETV name or logo does not imply approval or certification of this product nor does it make any explicit or implied warranties or guarantees as to product performance.

YSI Incorporated
Who's Minding the Planet?

YSI 6600 V2 Sensor Specifications

	Range	Resolution	Accuracy
ROX™ Optical Dissolved Oxygen* % Saturation	0 to 500%	0.1%	0 to 200%: ±1% of reading or 1% air saturation, whichever is greater; 200 to 500%: ±15% of reading
ROX™ Optical Dissolved Oxygen* mg/L	0 to 50 mg/L	0.01 mg/L	0 to 20 mg/L: ± 0.1 mg/L or 1% of reading, whichever is greater; 20 to 50 mg/L: ±15% of reading
Dissolved Oxygen** % Saturation ETV 6562 Rapid Pulse™ Sensor*	0 to 500%	0.1%	0 to 200%: ±2% of reading or 2% air saturation, whichever is greater; 200 to 500%: ±6% of reading
Dissolved Oxygen** mg/L ETV 6562 Rapid Pulse™ Sensor*	0 to 50 mg/L	0.01 mg/L	0 to 20 mg/L: ± 0.2 mg/L or 2% of reading, whichever is greater; 20 to 50 mg/L: ±6% of reading
Conductivity*** ETV 6560 Sensor*	0 to 100 mS/cm	0.001 to 0.1 mS/cm (range dependent)	±0.5% of reading + 0.001 mS/cm
Salinity	0 to 70 ppt	0.01 ppt	±1% of reading or 0.1 ppt, whichever is greater
Temperature ETV 6560 Sensor*	-5 to +50°C	0.01°C	±0.15°C
pH ETV 6561 Sensor*	0 to 14 units	0.01 unit	±0.2 unit
ORP	-999 to +999 mV	0.1 mV	±20 mV
Depth — Deep	0 to 656 ft, 200 m	0.001 ft, 0.001 m	±1 ft, ±0.3 m
Depth — Medium	0 to 200 ft, 61 m	0.001 ft, 0.001 m	±0.4 ft, ±0.12 m
Depth — Shallow	0 to 30 ft, 9.1 m	0.001 ft, 0.001 m	±0.06 ft, ±0.02 m
Depth — Vented Level	0 to 30 ft, 9.1 m	0.001 ft, 0.001 m	±0.01 ft, 0.003 m
Turbidity* ETV 6136 Sensor*	0 to 1,000 NTU	0.1 NTU	±2% of reading or 0.3 NTU, whichever is greater**
Nitrate/nitrogen****	0 to 200 mg/L-N	0.001 to 1 mg/L-N (range dependent)	±10% of reading or 2 mg/L, whichever is greater
Ammonium/ammonia/ nitrogen****	0 to 200 mg/L-N	0.001 to 1 mg/L-N (range dependent)	±10% of reading or 2 mg/L, whichever is greater
Chloride****	0 to 1000 mg/L	0.001 to 1 mg/L (range dependent)	±15% of reading or 5 mg/L, whichever is greater
Rhodamine*	0-200 µg/L	0.1 µg/L	±5% reading or 1 µg/L, whichever is greater

* Maximum depth rating for all optical probes is 200 feet, 61 m. Turbidity and Rhodamine are also available in a Deep Depth option (0 to 200 m)
** Rapid Pulse is only available on 6600 V2-2 (two optical ports version)
*** Report outputs of specific conductance (conductivity corrected to 25° C), resistivity, and total dissolved solids are also provided. These values are automatically calculated from conductivity according to algorithms found in *Standard Methods for the Examination of Water and Wastewater* (ed 1989)
**** Freshwater only. Maximum depth rating of 50 feet, 15.2 m. 6600 V2-2 has 3 ISE ports, not available on the 6600V2-4.
**In YSI AMCO-AEPA Polymer Standards

	Range	Detection Limit	Resolution	Linearity
Blue-Green Algae Phycocyanin*	~0 to 280,000 cells/mL† 0 to 100 RFU	~220 cells/mL§	1 cell/mL 0.1 RFU	$R^2 > 0.9999$**
Blue-Green Algae Phycoerythrin*	~0 to 200,000 cells/mL† 0 to 100 RFU	~450 cells/mL§§	1 cell/mL 0.1 RFU	$R^2 > 0.9999$***
Chlorophyll* ETV 6025 Sensor*	~0 to 400 µg/L 0 to 100 RFU	~0.1 µg/L§§§	0.1 µg/L Chl 0.1% RFU	$R^2 > 0.9999$****

* Maximum depth rating for all optical probes is 200 feet, 61 m. Also available in a Deep Depth option (0 to 200 m)
RFU = Relative Fluorescence Units

† Explanation of Ranges can be found in the 'Principles of Operation' section of the 6-Series Manual, Rev D

§ Estimated from cultures of *Microcystis aeruginosa*.
§§ Estimated from cultures *Synechococcus sp.*
§§§ Determined from cultures of *Isochrysis sp.* and chlorophyll *a* concentration determined via extractions.

** For serial dilution of Rhodamine WT (0-400 ug/l)
*** For serial dilution of Rhodamine WT (0-8 µg/L).
**** For serial dilution of Rhodamine WT (0-500 ug/L)

YSI 6600 V2 Sonde Specifications

Medium	Fresh, sea or polluted water	Software	EcoWatch*
Temperature — Operating	-5 to +50°C	Dimensions — Diameter	3.5 in, 8.9 cm
Temperature — Storage	-10 to +60°C	Dimensions — Length, no depth	19.6 in, 49.8 cm
		Dimensions — Length, with depth	21.6 in, 54.9 cm
		Weight	7 lbs, 3.18 kg (batteries installed, with depth)
Communications	RS-232, SDI-12	Power — External	12 V DC
		Power — Internal	8 C-size alkaline batteries

APPENDIX C. QA TAXONOMIST QUALIFICATIONS

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nixy consulting
3174 Rock City Rd, Nanaimo, BC, Canada V9T 1T4
phone: (250) 756-0956 cell: (250) 537-7176 email: nixy@telus.net

Statement of Qualifications: Nicky Haigh

Project: Taxonomic analysis of marine phytoplankton species, with an emphasis on species diversity.

Rates: US$40/hr

Services:

- Phytoplankton taxonomy, specializing in marine temperate harmful species
- Monitoring of algal populations and environmental parameters for finfish and shellfish aquaculture
- Education in phytoplankton taxonomy, enumeration, and environmental sampling

Past Projects:

- *Harmful Algae Monitoring Program (1999 to present):* working with the finfish aquaculture companies to monitor and mitigate harmful algal blooms in BC. Part of the mandate of the program is to monitor the phytoplankton species present in the marine environment of BC and build up a database of local phytoplankton species diversity and abundance, concentrating on, but not limited to, species found to be toxic to finfish. Weekly samples sent by the fish farmers are analysed for: identification of algal species to most practicable taxonomic level; enumeration of dominant species and any harmful species or species of concern to finfish aquaculture; overall plankton biomass (roughly approximated on a scale of 1 to 5); and approximation of proportion of planktonic biomass in five basic groups – diatoms, dinoflagellates, raphidophytes, other flagellates, and microzooplankton. Also in the HAMP mandate is the planning and conducting of annual workshops at farm sites to educate farm personnel on phytoplankton species identification. To assist in this training I have written the *HAMP Harmful Plankton Handbook*: a phytoplankton identification book published for the fish farmers and updated annually, with images and information on local algae species, concentrating on, but not limited to, species that are harmful to aquacultured fish in BC.
- *EUCFe 2006 Phytoplankton sample analysis (2008):* Analysis of phytoplankton samples from the Equatorial Pacific for Dr Diana Varela, University of Victoria (email: dvarela@uvic.ca).
- *Islands Scallops sample analysis (2006 to present):* Analysis of weekly phytoplankton samples for a shellfish aquaculture operation. Analysis consisted of: identification of species to most practicable taxonomic level; enumeration of

dominant species and any harmful species or species of concern to shellfish aquaculture; overall plankton biomass (roughly approximated on a scale of 1 to 5); and approximation of proportion of planktonic biomass in five basic groups – diatoms, dinoflagellates, raphidophytes, other flagellates, and microzooplankton.

- *Quinsam Plankton Project, Campbell River, BC, Canada (2007):* Helped to develop project to monitor phytoplankton spring bloom, zooplankton and juvenile coho salmon in the area of the Campbell River Estuary, to see if knowledge of plankton peaks can optimize timing of the release of coho salmon fry from the Quinsam Hatchery (Fisheries and Oceans Canada) and thus the survival of the coho salmon stocks in the Strait of Georgia, BC.

Education:

BSc, Combined Honours Biology and Oceanography, University of British Columbia 1994. Honours Thesis: *Varied Ichthyotoxic Effects of Six Strains of the Photosynthetic Dinoflagellate* Gyrodinium galatheanum *on the Threespine Stickleback* Gasterosteus aculeatus.

Certificate of Proficiency in Identification of Harmful Marine Microalgae. 2007. IOC Science and Communication Centre on Harmful Algae, University of Copenhagen.

List of publications:

Haigh, N. 2008. HAMP 2007 Annual Report. Client Report for Marine Harvest Canada, Mainstream Canada, Creative Salmon, Grieg Seafoods BC Ltd., Omega Pacific Aquaculture, and Sablefish Canada. 193pp.

Haigh, N. 2008. Harmful Plankton Handbook: HAMP 2008. 51pp. HAMP Publications, HAMPbook@telus.net.

Haigh, N., J.N.C. Whyte, and K.L. Sherry. 2004. Biological and oceanographic data from the Harmful Algae Monitoring Program associated with salmon farm sites on the west coast of Canada in 2003. Can. Data Rep. Fish. Aqu. Sci. No 1158.

Haigh, N., J.N.C. Whyte, and K.L. Sherry. 2004. Biological and oceanographic data from the Harmful Algae Monitoring Program associated with salmon farm sites on the west coast of Canada in 2001. Can. Data Rep. Fish. Aqu. Sci. No 1156.

Haigh, N., J.N.C. Whyte, and K.L. Sherry. 2004. Biological and oceanographic data from the Harmful Algae Monitoring Program associated with salmon farm sites on the west coast of Canada in 2000. Can. Data Rep. Fish. Aqu. Sci. No 1155.

Haigh, N., J.N.C. Whyte, and K.L. Sherry. 2004. Biological and oceanographic data from the Harmful Algae Monitoring Program associated with salmon farm sites on the west coast of Canada in 1999. Can. Data Rep. Fish. Aqu. Sci. No 1154.

Waters, R., N. Haigh, J.N.C. Whyte, and C.D. Levings. 2001. Synoptic investigation for algae in ballast water and sediments of ships using selected British Columbia ports. Can. Data Rep. Fish. Aquat. Sci. 1083.

Whyte J.N.C., N. Haigh, N.G. Ginther, and L. Keddy. 2001. First record of blooms of *Cochlodinium* sp. (Gymnodiniales, Dinophyceae) causing mortality to aquacultured salmon on the west coast of Canada. Phycologia 40: 298-304.

APPENDIX D. EXTERNAL QA TAXONOMY DATA TEMPLATE

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King County Puget Sound Phytoplankton QC Data

Sample Information

Locator	Sample ID	Depth	Sample Date	Date Processed

Results

Lowest Taxon	Qualifier	Comments